

What is claimed is:

- 5     1. A method for introducing an agent into a cell comprising providing a preparation comprising the cell and agent, and applying the nanosecond pulse electric fields to said preparation, which facilitates the entry of the agent into the nucleus.
2. The method of claim 1, wherein the agent is selected from the group comprising drugs, nucleic acids, protein, peptides, and polypeptides.
- 10    3. The method of claim 2, wherein the drug is an antibiotic.
4. The method of claim 2, wherein the drug is a chemotherapeutic agent.
5. The method of claim 4, wherein the chemotherapeutic agent is selected from the group comprising bleomycin, daunomycin, 5-FU, cytosine arabinoside, colchicine, cytochalasin B, daunorubicin, neocarzinostatin, suramin, doxorubicin, carboplatin, taxol, mitomycin C,  
15    vincristine, vinblastine, methotrexate, and cisplatin, and suitable combinations thereof.
6. The method of claim 2, wherein the agent is a nucleic acid.
7. The method of claim 6, wherein the nucleic acid is selected from the group comprising DNA, cDNA, and RNA.
8. The method of claim 6, wherein the nucleic acid encodes a homologous or heterologous gene  
20    product.
9. The method of claim 8, wherein the cell is transfected with the nucleic acid so that the gene product is expressed in the cell.
10. The method of claim 8, wherein the nucleic acid is an expression vector.
11. The method of claim 10, wherein the expression vector contains a homologous or heterologous  
25    nucleic acid encoding a gene product operably linked to a suitable promoter sequence.

12. The method of claim 8, wherein the gene product is expressed in the cell.
13. The method of claim 6, wherein the nucleic acid modulates the expression of a gene.
14. The method of claim 13, wherein the nucleic acid provides gene therapy.
15. The method of claim 6, wherein the nucleic acid modulates cell proliferation.
- 5 16. The method of claim 6, wherein the nucleic acid elicits an immune response.
17. The method of claim 1, wherein the agent is a vaccine.
18. The method of claim 2, wherein the polypeptide is a hormone, a cytokine, a lymphokine, a growth factor, or a combination thereof.
19. The method of claim 2, wherein the polypeptide is an antigen.
- 10 20. The method of claim 2, wherein the polypeptide is an antibody.
21. The method of claim 1, wherein the agent is a cytotoxic agent.
22. The method of claim 21, wherein the cytotoxic agent is selected from the group comprising ricin, abrin, diphtheria toxin, and saporin.
- 15 23. The method of claim 1, wherein the cell is selected from the group comprising eukaryotic cells, prokaryotic cells, fat cells, bone cells, vascular cells, muscle cells, cartilage cells, bacterial cells, and combinations thereof.
24. The method of claim 1, wherein the cell is a cancer cell.
- 20 25. The method of claim 24, wherein the cancer cell is selected from the group of cancers comprising adenocarcinoma, squamous carcinoma, carcinoma of the organs, sarcoma, chondrosarcoma, melanosa sarcoma, leukemia, lymphoma, acute lymphomastic leukemia, acute myelogenous leukemia, non-Hodgkin's lymphoma, Burkitt's lymphoma, B-cell lymphoma, and T-cell lymphoma.

26. The method of claim 25, wherein the cancer cell is a leukemia or fibrosarcoma cell.
27. The method of claim 1, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.
28. The method of claim 27, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 500 nanoseconds.
29. The method of claim 28, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.
30. The method of claim 29, wherein the nanosecond pulse electric field has a pulse duration of about 10 nanoseconds to about 60 nanoseconds.
31. The method of claim 1, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.
32. The method of claim 31, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.
33. The method of claim 32, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 250 kV/cm.
34. A method of enhancing gene expression in a cell comprising providing a preparation comprising the cell and the nucleotide sequence to be delivered into the cell, and applying nanosecond pulse electric fields to said preparation, wherein said application facilitates the entry of the agent into the nucleus.
35. The method of claim 34, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.
36. The method of claim 35, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 500 nanoseconds.
37. The method of claim 36, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.

38. The method of claim 37, wherein the nanosecond pulse electric field has a pulse duration of about 10 nanoseconds to about 60 nanoseconds.
39. The method of claim 34, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.
- 5 40. The method of claim 39, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.
41. The method of claim 40, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 250 kV/cm.
- 10 42. A method of enhancing gene expression in a cell comprising transfecting said cell with the desired gene and applying nanosecond pulse electric fields to said cell.
43. The method of claim 42, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.
44. The method of claim 43, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 500 nanoseconds.
- 15 45. The method of claim 44, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.
46. The method of claim 45, wherein the nanosecond pulse electric field has a pulse duration of about 10 nanoseconds to about 60 nanoseconds.
- 20 47. The method of claim 42, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.
48. The method of claim 47, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.
49. The method of claim 48, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 250 kV/cm.

50. The method of claim 42, wherein the cell is transfected by electroporation.
51. The method of claim 42, wherein the cell is transfected by the use of lipid vesicles.
52. The method of claim 42, wherein the cell is transfected by the use of viral vectors.
53. The method of claim 52, wherein said viral vectors are selected from the group comprising an  
5 adenovirus vector, a herpes virus vector, a vaccinia vector, and a retroviral vector.
54. The method of claim 42, wherein the cell is transfected by co-precipitation of said gene with calcium phosphate.
55. The method of claim 42, wherein the cell is transfected by co-precipitation of said gene with dextran.
- 10 56. A method of enhancing gene expression in a cell comprising applying one or more long pulses to said cell and applying one or more nanosecond pulse electric field pulses to said cell.
57. The method of claim 56, wherein said long pulses has a duration of about 1 microsecond to about 20 milliseconds.
58. The method of claim 56, wherein said nanosecond electric field pulse has a duration of about 1  
15 nanosecond to about 300 nanoseconds.
59. A method of enhancing delivery of drugs to tumors or other tissues comprising applying nanosecond pulse electric fields to said tumors or other tissues.
60. The method of claim 59, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.
- 20 61. The method of claim 60, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 500 nanoseconds.
62. The method of claim 61, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.

63. The method of claim 62, wherein the nanosecond pulse electric field has a pulse duration of about 10 nanoseconds to about 60 nanoseconds.
64. The method of claim 59, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.
- 5 65. The method of claim 64, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.
66. The method of claim 65, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 250 kV/cm.
67. A pulse generator for generating electrical pulses comprising:
- 10       a first circuit for generating a first pulse having a long duration and low voltage amplitude;
- a second circuit for generating a second pulse having a short duration and high voltage amplitude; and
- a control circuit for controlling the timing of said first circuit and said second circuit to respectively generate said first pulse and said second pulse.
- 15 68. The pulse generator of claim 67, wherein said first pulse has a duration of 0.1 millisecond to 20 milliseconds.
69. The pulse generator of claim 67, wherein said first pulse has a duration of 0.001 millisecond to 30 milliseconds.
70. The pulse generator of claim 67, wherein said second pulse has a duration of 1 nanosecond to 300  
20 nanoseconds.
71. The pulse generator of claim 67, wherein said second pulse has a duration of 1 nanosecond to 1000 nanoseconds.
72. The pulse generator of claim 67, wherein said first pulse has an electric field strength of 0.1 kV/cm to 5 kV/cm.

73. The pulse generator of claim 67, wherein said first pulse has an electric field strength of 0.1 kV/cm to 1 kV/cm.
74. The pulse generator of claim 67, wherein said second pulse has an electric field strength of 10 kV/cm to 350 kV/cm.
- 5 75. The pulse generator of claim 67, wherein said second pulse has an electric field strength of 1 kV/cm to 1000 kV/cm.
76. The pulse generator of claim 67, wherein said control circuit allows an interval of 1 millisecond to 5 hours between said first pulse and said second pulse.
- 10 77. The pulse generator of claim 67, wherein said control circuit allows an interval of 1 millisecond to 24 hours between said first pulse and said second pulse.
78. The pulse generator of claim 67, further comprising a delivery apparatus for delivering said first pulse and said second pulse to a load.
79. The pulse generator of claim 78, wherein said delivery apparatus comprises a pair of electrodes.
- 15 80. The pulse generator of claim 78, wherein the load comprises at least one of cells in suspension and biological tissue.
81. The pulse generator of claim 67, wherein said first circuit comprises:
- a high voltage power supply;
  - a charging resistor coupled to said high voltage power supply;
  - a capacitor coupled at a first end to said charging resistor and coupled at a second end to a
  - 20 load; and
  - a transistor for controlling electrical discharge of said capacitor to the load.
82. The pulse generator of claim 81, wherein said transistor is responsive to at least one command from said control circuit to control the electrical discharge of said capacitor.

83. The pulse generator of claim 81, wherein said transistor has a low forward voltage for handling sustained high currents without suffering thermal damage.
84. The pulse generator of claim 81, wherein said transistor is an insulated gate bipolar transistor.
- 5 85. The pulse generator of claim 67, further comprising a first switch coupled to and controlled by said control circuit, said first switch being operable to couple and decouple the first circuit from a load.
86. The pulse generator of claim 85, wherein said first switch is at least one of a mechanical switch and a vacuum switch.
87. The pulse generator of claim 85, wherein said first switch is a magnetic switch.
- 10 88. The pulse generator of claim 85, further comprising a first trigger unit coupled to said control circuit for actuating said first switch responsive to a first command from said control circuit.
89. The pulse generator of claim 67, wherein said second circuit comprises:
- a high voltage power supply;
- a charging resistor coupled to said high voltage power supply; and
- 15 a transmission line coupled at a first end to said charging resistor and coupled at a second end to a load, said transmission line electrically discharging into the load.
90. The pulse generator of claim 89, wherein said transmission line is a Blumlein configuration transmission line.
- 20 91. The pulse generator of claim 89, further comprising a second switch coupled to and controlled by said control circuit, said second switch being operable to discharge the transmission line.
92. The pulse generator of claim 91, wherein said second switch is a spark gap switch.
93. The pulse generator of claim 91, further comprising a second trigger unit coupled to said control circuit for actuating said second switch responsive to a second command from said control circuit.
94. A pulse generator for generating electrical pulses comprising:



first generator means for generating a first pulse having a long duration and low voltage amplitude;

second generator means for generating a second pulse having a short duration and high voltage amplitude; and

5 control means for controlling timing of the pulses generated by said first generator means and said second generator means.

95. The pulse generator of claim 94, wherein said first generator means generates said first pulse for a duration of 0.1 millisecond to 20 milliseconds.

10 96. The pulse generator of claim 94, wherein said first generator means generates said first pulse for a duration of 0.001 millisecond to 30 milliseconds.

97. The pulse generator of claim 94, wherein said second generator means generates said second pulse for a duration of 1 nanosecond to 300 nanoseconds.

98. The pulse generator of claim 94, wherein said second generator means generates said second pulse for a duration of 1 nanosecond to 1000 nanoseconds.

15 99. The pulse generator of claim 94, wherein said first pulse has an electric field strength ranging from 0.1 kV/cm to 5 kV/cm.

100. The pulse generator of claim 94, wherein said first pulse has an electric field strength ranging from 0.1 kV/cm to 1 kV/cm.

20 101. The pulse generator of claim 94, wherein said second pulse has an electric field strength ranging from 10 kV/cm to 350 kV/cm.

102. The pulse generator of claim 94, wherein said second pulse has an electric field strength ranging from 1 kV/cm to 1000 kV/cm.

103. The pulse generator of claim 94, wherein said control circuit allows an interval of 1 millisecond to 5 hours between said first pulse and said second pulse.

104. The pulse generator of claim 94, wherein said control circuit allows an interval of 1 millisecond to 24 hours between said first pulse and said second pulse.

105. The pulse generator of claim 94, further comprising delivery means for delivering said first pulse and said second pulse to a load.

5 106. The pulse generator of claim 105, wherein the load comprises at least one of cells in suspension and biological tissue.

107. A method of enhancing gene expression using a pulse generator, the method comprising the sequential, the non-sequential, and the sequence independent steps of:

10 triggering a first pulse having a long duration and low voltage amplitude from a first circuit of the pulse generator;

delivering the first pulse to at least one cell to cause electroporation at the plasma membrane of the at least one cell;

triggering a second pulse having a long duration and low voltage amplitude from a second circuit of the pulse generator; and

15 delivering the second pulse to the at least one cell to cause electroporation at the nuclear membrane of the at least one cell.

108. The method of claim 107, wherein the step of triggering a first pulse further comprises the steps of:

charging a capacitor; and

20 triggering a high voltage, high current transistor to initiate discharge of the charge accumulated in the capacitor;

wherein the charge is the first pulse delivered to the at least one cell.

109. The method of claim 108, further comprising a step of triggering the transistor to stop discharge of the capacitor.

25 110. The method of claim 107, wherein the step of triggering a second pulse further comprises the steps:

charging a transmission line; and

triggering a high voltage switch to initiate discharge of the charge accumulated in the transmission line;

wherein the charge is the second pulse delivered to the at least one cell.

5 111. The method of claim 110, further comprising a step of triggering the high voltage switch to stop discharge of the transmission line.

112. The method of claim 107, further comprising a step of waiting a predetermined interval prior to delivering the second pulse.

10 113. The method of claim 112, wherein the predetermined interval is based on diffusion of polynucleotides from the plasma membrane to the nuclear membrane of the at least one cell.

114. The method of claim 112, wherein the predetermined interval ranges from 1 millisecond to 24 hours.

115. A method of enhancing gene expression in a cell using a multi-pulse generator, the method comprising the sequential, the non-sequential, and the sequence independent steps of:

15 charging a capacitor;

triggering a high voltage, high current transistor to initiate discharge of the charge accumulated in the capacitor into at least one cell to cause electroporation at the plasma membrane of the at least one cell;

20 triggering the high voltage, high current transistor to stop the discharge of the capacitor after a predetermined long duration;

actuating a switch to decouple the capacitor from the at least one cell;

charging a transmission line;

25 triggering a high voltage switch to initiate discharge of the charge accumulated in the transmission line into the at least one cell to cause electroporation at the nuclear membrane of the at least one cell; and

triggering the high voltage switch to stop discharge of the transmission line after a predetermined short duration.

116. A multi-pulse generator for causing electroporation at both a cellular plasma membrane and a nuclear membrane, said multi-pulse generator comprising:

5 means for triggering a first pulse having a long duration and low voltage amplitude;

means for delivering said first pulse to at least one cell, said first pulse causing electroporation at the plasma membrane of the at least one cell;

means for triggering a second pulse having a short duration and high voltage amplitude; and

10 means for delivering the second pulse to the at least one cell, said second pulse causing electroporation at the nuclear membrane of the at least one cell.

117. A multi-pulse generator for enhancing gene expression in a cell, said multi-pulse generator comprising:

means for accumulating a charge;

15 means for selectively discharging the accumulated charge into at least one cell to cause electroporation at the plasma membrane of the at least one cell, and terminating discharge of the accumulated charge;

means for charging a transmission line; and

20 means for selectively discharging said transmission line into the at least one cell to cause electroporation at the nuclear membrane of the at least one cell, and terminating discharge of the transmission line.

118. A dual-pulse generator for enhancing gene expression in a cell, said dual-pulse generator comprising:

a first pulse generator for generating a first pulse having a long duration and low voltage amplitude, said first pulse causing electroporation of the cellular plasma membrane of the cell;

25 a second pulse generator for generating a second pulse having a short duration and high voltage amplitude, said second pulse causing electroporation of the nuclear membrane of the cell; and

a control circuit for controlling timing of pulses generated by said first pulse generator and said second pulse generator.

119. A dual-pulse generator for enhancing gene expression in a cell, the dual-pulse generator comprising:

first generator means for generating a first pulse having a long duration and low voltage amplitude, said first pulse causing electroporation of the cellular plasma membrane of the cell;

5 second generator means for generating a second pulse having a short duration and high voltage amplitude, said second pulse causing electroporation of a nuclear membrane of the cell; and

control means for controlling timing of the pulses generated by said first generator means and said second generator means.

120. A dual-pulse generator for enhancing gene expression in a cell, the dual-pulse generator  
10 comprising:

a first high voltage power supply;

a first charging resistor coupled to said first high voltage power supply;

a capacitor coupled at a first end to said first charging resistor and coupled at a second end to a load;

15 a transistor coupled to the second end of the first charging resistor and the first end of the capacitor, said transistor controlling electrical discharge of the capacitor to said load;

a second high voltage power supply;

a second charging resistor coupled to said second high voltage power supply;

20 a transmission line coupled at a first end to said second charging resistor and coupled at a second end to the load;

a control circuit for controlling electrical discharge of said capacitor and said transmission line;

a first switch for selectively coupling and decoupling said capacitor from the load;

25 a first trigger unit coupled to said control circuit and said first switch, said first trigger unit actuating said first switch responsive to one or more commands received from said control circuit;

a second switch for selectively discharging said transmission line;

a second trigger unit coupled to said control circuit and said second switch, said second trigger unit actuating said first switch responsive to one or more commands received from said control circuit; and

5 a delivery apparatus for delivering the electrical discharges from said capacitor and said transmission line to the load.

121. A method of enhancing gene expression using a dual-pulse generator, the method comprising the sequential, the non-sequential, and the sequence independent steps of:

triggering a first pulse type from the dual-pulse generator, the first pulse type having a long duration and low voltage amplitude;

10 delivering the first pulse type to at least one cell to cause causes electroporation at the plasma membrane of the at least one cell;

waiting a predetermined time interval;

triggering a second pulse type from the dual-pulse generator, the second pulse type having a short duration and high voltage amplitude; and

15 delivering the second pulse type to the at least one cell to cause electroporation at the nuclear membrane of the at least one cell.

122. A method of applying a nanosecond pulse electric field to a patient in need thereof.

123. The method of claim 122, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.

20 124. The method of claim 123, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.

125. The method of claim 122, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.

25 126. The method of claim 125, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.

127. The method of claim 122 wherein said patient has cancer.

128. A method of enhancing gene expression in a cell comprising applying a nanosecond pulse electric field to said cell.

129. The method of claim 128, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 1000 nanoseconds.

130. The method of claim 129, wherein the nanosecond pulse electric field has a pulse duration of about 1 nanosecond to about 300 nanoseconds.

5 131. The method of claim 128, wherein the nanosecond pulse electric field has an electric field intensity from about 1 kV/cm to about 1000 kV/cm.

132. The method of claim 131, wherein the nanosecond pulse electric field has an electric field intensity from about 10 kV/cm to about 350 kV/cm.